EXHIBIT B

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Mar Tormo
Ana M. Tari
Gabriel Lopez-Berestein

Serial No.: 08/726,211

Filed: October 4, 1996

For: INHIBITION OF BCL-2 PROTEIN EXPRESSION BY LIPOSOMAL ANTISENSE OLIGODEOXYNUCLEOTIDES

Group Art Unit: 1636

Examiner: R. Schwartzman

Atty. Dkt. No.: UTXC:504/STA

CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence is being hand delivered for filing to: Examiner R. Schwartzman, Group 1636, Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

Date _____ Signature _____

DECLARATION OF DRS. GABRIEL LOPEZ-BERESTEIN AND ANA M. TARI

We, Dr. Gabriel Lopez-Berestein and Dr. Ana M. Tari, inventors of the present invention, hereby declare as follows:

1. We, along with Dr. Mar Tormo, are co-inventors of the subject matter of the captioned patent application USSN 08/726,211.

2. We understand that the patent examiner in charge of assessing the patentability of the instant application has rejected the claims of this application. We are providing this declaration to submit additional data relating to this application.

3. We have experimentally demonstrated that DOPC lipids associated with P-ethoxy antisense BCL-2 oligonucleotides are effective in delivering the antisense oligonucleotides to cells following the methods disclosed in patent application USSN 08/726,211. These neutral lipid associations also selectively inhibit cell growth as only lipid:BCL-2 antisense oligonucleotide associations, and not lipid:BCL-2 control oligonucleotide associations, induce cell growth inhibition and decreased cell viability relative to untreated cells.

4. Because other investigators have used lipid associations carrying a net positive or negative charge to deliver antisense oligonucleotides, we have examined the effects of lipid charge on antisense delivery and activity employing the methods described in the present application. In this study, 30 mole percent of a negatively-charged lipid (DMPG) or a positively-charged lipid (DC-CHOL) were added into lipid:BCL-2 antisense or lipid:BCL-2 control oligonucleotide associations containing a neutral lipid (70 mole percent, DOPC). The control oligonucleotides are scrambled BCL-2 oligonucleotides and these lipid:oligonucleotide associations were delivered to cells in the same manner as disclosed in patent application USSN 08/726,211. The cell growth and viability observed with these charged lipid associations were compared with that observed with lipid:BCL-2 antisense and control oligonucleotide associations containing only a neutral lipid.

5. Surprisingly, we found that both the negatively- and positively-charged lipid:oligonucleotide associations were very toxic to cells and toxicity could be seen as low as 2 μM concentrations. The growth inhibition and cytoxicity caused by such charged lipid:oligonucleotide associations is also non-specific. We observed no significant difference between the viability of cells exposed to the DOPC:DMPG or DOPC:DC-CHOL lipid:BCL-2 antisense oligonucleotide associations and the corresponding DOPC:DMPG or DOPC:DC-CHOL lipid:BCL-2 control oligonucleotide associations whereas only the cells exposed to DOPC lipid:antisense oligonucleotide associations, and not the DOPC lipid:control oligonucleotide associations, exhibited decreased viability in relation to the viability of untreated cells, as indicated in the table below.

% OF VIABILITY*						
	DOPC (100%)		DOPC-DMPG (7:3 mole ratio)		DOPC-DC-CHOL (7:3 mole ratio)	
μM of oligonucleotide	Control	Antisense	Control	Antisense	Control	Antisense
2	108	103	55.3	37.0	77.9	37.0
4	125	91	46.0	18.1	56.3	10.1
6	128	94	26.1	31.0	24.2	12.9
8	135	89	19.5	22.9	23.4	10.1
10	134	86	19.5	32.0	7.9	19.9
12	123	75	20.9	32.0	14.5	18.9

* In relation to untreated cells.

6. These results are surprising and unexpected as this indicates that only the neutral DOPC lipids and not charged lipids can be used to safely and effectively deliver antisense oligonucleotides to cells and thereby achieve selective cytotoxicity and cell growth inhibition.

7. In view of the above-described studies and those disclosed in the specification, it is clear that the methods and compositions employing antisense BCL-2 in association with neutral lipids disclosed and claimed in this application have surprising and unexpected properties with respect to similar methods and compositions employing antisense BCL-2 lipid associations wherein the lipid association has an overall positive or negative charge.

8. We declare that all statements made of our own knowledge are true and that all statements made on information are believed to be true; and, further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Codes, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Signed:

Gabriel Lopez-Berestein

Dated: June 10, 1998

Ana M. Tari

Dated: June 10, 98